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Express Mail Label No.

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☐ *Additional inventors are being named on the* 0 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

COMBINED EXPONENTIAL AND LINEAR AMPLIFICATION

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Respectfully submitted,

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Date 24 OCT 02

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Provisional Patent Application of

Guoliang Fu

For

COMBINED EXPONENTIAL AND LINEAR AMPLIFICATION

Abstract

Methods are provided for sensitive detection and quantitation of nucleic acids. Methods are further provided for genotyping. Methods of invention involve nicking target sequence by a nuclease, extension from nicking site by a strand-displacing DNA polymerase, nicking and extension again to generate multiple copies of single stranded nucleic acids, which can serve as primer to anneal to a new template, prime extension and nicking. DNAzyme mediated detection method is also provided for detecting one type of the amplification end product - single stranded nucleic acids.

References Cited

U.S. Patent documents

6,316,229	Nov., 2001	Lizardi, et al.
5,011,769	Apr., 1991	Duck, et al.
6,271,002	Aug., 2001	Linsley, et al.
6,297,365	Oct., 2001	Adams, et al.
6,300,068	Oct , 2001	Burg, et al.

Foreign Patent Documents

WO0109376	Feb., 2001	Cardy, et al.

BACKGROUND OF THE INVENTION

This invention relates to the filed of nucleic acid amplification and detection. More particularly, the invention provides methods, compositions and kits for amplifying (i.e., making multiple copies) nucleic acid sequences and for detecting amplified sequences, which involve target initiated nucleic acid polymerization, chain reaction cascade and DNAzyme mediated detection.

A number of methods have been developed which permit the implementation of extremely sensitive diagnostic assays based on nucleic acid detection. They fall into two classes, enabling either target or signal amplification. Target amplification methods include the polymerase chain reaction (PCR), ligase chain reaction (LCR), self-sustained sequence replication (3 SR), nucleic acid sequence based amplification (NASBA), and strand displacement amplification (SDA). Signal amplification technologies include branched DNA (bDNA), hybrid capture, and cleavase, and measure nucleic acid targets by amplification of a surrogate marker. Rolling circle amplification (RCA) is a newer method that performs either target or signal amplification (Birkenneyer and Mushahwar, J. Virological Methods, 35·117-126 (1991); Landegren, Trends Genetics, 9:199-202 (1993); Schweitzer and Kingsmore, Current opinion in Biotechnology, 12 21-27 (2001)).

All of these methods are sensitive and compatible with many detection techniques, such as fluorescence, chemiluminescence, or gel electrophoresis. The PCR method can detect about 100 or less than 100 target molecules; other method's detection limits are within a range of several thousands or higher than several thousands target molecules. All of them have relatively low precision in quantitative measurements, especially for detection of low concentration of target molecules.

PCR remains the most widely used DNA amplification and quantitation method. Food and Drug Administration (FDA) has approved diagnostic kits (Roche) that use PCR for the detection and quantitation of HIV, Mycobacterium tuberculosis, and Chlamydia trachomatis. However, PCR in general suffer from several limitations that are well-

known in the art, such as requirement of expensive thermal cyclers, easy contamination, difficulty of quantification, amplification with different efficiencies for different DNAs, and limited multiplexing.

Current technologies for quantitative profiling of mRNA/cDNA levels in biological samples involve the use of either cDNA arrays (Schena et al., Proc. Natl Acad. Sci. USA, 91:10614-10619 (1994)) or high density oligonucleotide arrays (Lockhart et al, Nature Biotechnology, 14.1675-1680 (1996)). In the case of the cDNA arrays by Schena et al, the detection of a single molecular species in each element of the array requires the presence of at least 100,000 bound target molecules. In the case of the DNA chip arrays used by Lockhart et al, the detection limit for hybridized RNA is of the order of 2000 molecules.

Current technologies for detection of mutations in DNA include cloning and genetic screens, DNA sequencing (with or without cloning), Single Strand Conformational Polymorphism analysis (SSCP), Multiple Allele-Specific Detection Assay (MASDA), oligonucleotide arrays (DNA chips, such as Affymetrix), and ASO-PCR, or PCR plus genetic bit analysis with sequencing primers. Methods of detecting nucleotide sequences by ligating together two probes which hybridize to adjacent sequences in the target nucleic acid molecule are described in U.S. Pat. Nos. 4,883,750, 5,242,794, and 5,521,065, all to Whiteley et al. These methods do not involve replication or other amplification of the signal generated by ligation. Of all these methods, only cloning and genetic screens, or cloning followed by DNA sequencing are capable of detecting somatic mutations that may occur at a level of one DNA strand in 10,000 wild type strands.

Single nucleotide polymorphisms (SNPs) are the foundation of powerful complex trait and pharmacogenomic analysis, The availability of large SNP database, however, has emphasized a need for inexpensive SNP genotyping methods of commensurate simplicity, robustness, and scalability. In general, current methods require pre-amplification of genomic DNA, followed by SNP genotyping with an allele

discrimination method, such as DNA cleavage, ligation, single base extension or hybridization. Current methods are limited either by expense, inaccuracy, consumption of sample DNA, or lack of scalability (Faruqi et al BMC Genomics (2001) 2:4). Accordingly, there is a need for nucleic acid detection methods that are both sensitive and quantitative.

It is therefore an object of the disclosed invention to provide a method of detecting nucleic acid in little amount such as one molecule.

It is another object of the disclosed invention to provide a method of determining the amount of specific target nucleic acid sequences present in a sample where the number of signals measured is proportional to the amount of a target sequence in a sample and where the ratio of signals measured for different target sequences substantially matches the ratio of the amount of the different target sequences present in the sample.

It is another object of the disclosed invention to provide a method of detecting and determining the amount of multiple specific target nucleic acid sequences in a single sample where the ratio of signals measured for different target nucleic acid sequences substantially matches the ratio of the amount of the different target nucleic acid sequences present in the sample.

It is another object of the disclosed invention to provide a method of detecting the presence of single copies of target nucleic acid sequences. It is another object of the disclosed invention to provide a method of detecting the presence of target nucleic acid sequences representing individual alleles of a target genetic element.

It is another object of the disclosed invention to provide a method for detecting, and determining the relative amounts of, multiple molecules of interest in a sample.

It is another object of the disclosed invention to provide a method of high throughput genotyping SNP

It is another object of the disclosed invention to provide a method of detecting single stranded DNA end products by DNAzyme mediated cleavage of RNA or DNA-RNA chimera substrates.

SUMMARY OF THE INVENTION

Disclosed are compositions and methods for amplifying and detecting minute amount of nucleic acid, for gene expression profiling and for genotyping. The methods of invention make use of special designed oligonucleotide, referred to as "amplification repeat templates" (ART). The disclosed methods are highly sensitive, allowing detection of single molecule or gene expression profiling in single cell. This is accomplished through combined exponential and linear amplification (CELA) which allows production of numerous copies of single stranded primer-probe sequences (PPS) in a single, isothermal reaction. A single round of amplification using CELA results in a large amplification of the PPS, orders of magnitude greater than a single cycle of PCR replication and other amplification techniques in which each cycle is limited to a doubling of the number of copies of a target sequence.

The disclosed methods involve nicking target nucleic acid by a nuclease, for example restriction enzyme or RNase H, extension from nicking site by a strand-displacing DNA polymerase, nicking and extension again to generate multiple copies of single stranded primer-probe DNAs or to generate multiple copies of single stranded primer-probe RNA transcripts. The single stranded primer-probe sequences serve as primer to anneal to a new ART and prime extension and nicking. Exponential amplification takes place during repeated replication of primer-probes which anneal to new ARTs and prime new primer-probe generation. Linear amplification takes place when all ART molecules hybridize to primer-probes and become double stranded.

Following nucleic acid amplification in the disclosed methods, the amplified double stranded sequences, single stranded sequence and pyrophosphate (PPI) can be detected and quantified using any of the conventional detection systems for nucleic acids such as detection of fluorescent labels, enzyme-linked detection systems, microarray hybridization, capillary and gel electrophoresis, fluorescence polarization, mass spectrometry, Fluorescence Resonance Energy Transfer (FRET), Time-resolved fluorescence detection, electrical detection, and luminescence detection.

The invention also provides a detection method using DNAzyme The amplification repeat template harbors the complementary (antisense) sequence of a DNAzyme in its template repeat portions. During amplification, single-stranded PPS are produced that contain active (sense) copies of DNAzymes that cleave a reporter substrate included in the reaction mixture.

Major advantages of these methods are that all reagents are in single tube, the amplification operation is isothermal, and signals are strictly quantitative because the amplification reaction is combined linear and exponential and is catalyzed by a highly processive enzymes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1 is a diagram of examples of amplification repeat templates (ART) hybridized to a target sequence

FIG. 2 is a diagram of an example of CELA A target DNA sequence is hybridized to target complementary portion of amplification repeat templates (ART). The double stranded DNA is nicked on the target strand by a restriction enzyme digestion. By strand-displacing polymerase extension of nicked strand, and subsequent repeat nicking and extension, multiple copies of PPS are generated, which can then anneal to a new ART, prime new extension, nicking, and generation of new PPA. The resulting amplified end

products including double stranded DNA, single stranded PPS and PPI are then subjected to detection.

FIG.3 is a diagram of examples of amplification repeat templates (ART) hybridized to a target RNA sequence.

FIG. 4 is a diagram of an example of CELA. A target RNA sequence is hybridized to target complementary portion of amplification repeat templates (ART). The double stranded RNA/DNA hybrid is nicked on the RNA strand by RNase H digestion. By strand-displacing polymerase extension of nicked strand, and subsequent repeat nicking and extension, multiple copies of PPS are generated, which can then anneal to a new ART, prime new extension, nicking, and generation of new primer-probes. The resulting amplified end products including double stranded DNA, single stranded PPS and PPI are then subjected to detection

FIG.5 is a diagram of examples of amplification repeat templates (ART) hybridized to target sequences. ARTs contain RNA polymerase promoter portions

FIG. 6 is a diagram of an example of CELA. A target RNA or DNA sequence is hybridized to target complementary portion of amplification repeat templates (ART), which contains a RNA polymerase promoter portion. The double stranded RNA/DNA or DNA/DNA hybrid is nicked on the target strand by an enzymatic digestion. A strand-displacing polymerase extend the 3' end of nicked strand. . Once the promoter portion becomes double stranded, the RNA polymerase acts on the promoter and generates multiple copies of RNA transcripts, i.e. single stranded primer-probe RNA sequences, which then anneal to a new ART, prime new extension, and generation of new primer-probes. The resulting amplified end products including double stranded polynucleotides, single stranded PPS and PPI are then subjected to detection.

FIG. 7 shows variant forms of amplification repeat template (ART) which can be used for genotyping SNPs.

FIG. 8 is a diagram of an example of CELA for genotyping SNPs. A target RNA or DNA sequence is allele-specifically hybridized to target complementary portions of amplification repeat templates (ART), while a helper-primer anneals to helper-primer initiation portion of ART and also anneals to a region of target molecule which is adjacent to the hybridization region of ART's target complementary region. A DNA polymerase extends 3' end of helper-primer on ART template to create double stranded functional type IIS restriction site. A type IIS restriction enzyme (for example Fok I) nicks target DNA or RNA, because its opposite strand - ART contains modified nucleotides which render it resistant to cleavage. The strand-displacing DNA polymerase extends the 3' end of nicked strand. Through subsequent repeat nicking and extension, multiple copies of PPS are generated, which can then anneal to free ARTs, prime new extension, nicking, and generation of new PPS. The resulting amplified end products including double stranded polynucleotides, single stranded PPS and PPI are then subjected to detection.

FIG 9 shows examples of detection methods.

FIG. 10 is a diagram of an example of DNAzyme mediated detection of end products – single stranded PPS. The template repeat portion of ART contains a complementary (antisense) sequence of a DNAzyme, for example 10-23 DNAzyme. During the chain reaction cascade of amplification, single-stranded PPS are produced that contain active (sense) copies of DNAzymes. The DNA enzyme binds an RNA or DNA-RNA chimeric reporter substrate which contain fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site. Cleavage of this reporter substrate produces an increase in fluorescence that is indicative of successful amplification of the target initiated single stranded nucleic acids

DETAILED DESCRIPTION OF THE INVENTION

An example of a method of invention is outlined below.

1. The complementary portion of amplification repeat templates is hybridized to a target sequence
2. The target sequence which is hybridized to the complementary portion of amplification repeat templates is nicked by an enzymatic digestion.
3. The 3'end of nicked target sequence is extended by a polymerase on the template of ART
4. The extended strand is nicked, and extended again, this process is repeated to generate multiple copies of single stranded sequence complementary to a portion of ART. The new nicking site can be the same in the step 2 (FIG. 2), or another site created in a new place on the ART (FIG. 4). Alternatively, if the ART contains a RNA polymerase promoter sequence, multiple copies of single stranded RNA will be generated from the template repeat portion of ART in the presence of a cognate RNA polymerase and appropriate condition (FIG. 6). The single stranded DNA or RNA generated from this operation is referred to as primer-probe sequence (PPS).
5. The single stranded PPS from step 4 will act as primer to anneal to an upstream unit of template repeat portions of a free ART and is extended by a polymerase. This extended sequence is nicked and extended again to generate multiple copies of PPS (FIG. 2 and FIG. 4). Alternatively, if the ART contains a RNA polymerase promoter sequence, multiple copies of single stranded RNA primer-probe will be generated from the template repeat portion of ART in the presence of a cognate RNA polymerase and appropriate condition (FIG. 6)

Because all reagents are in a single tube, all above steps will occur simultaneously, there is no distinct boundary between the steps. As long as there is free ART which is not hybridized to a PPS), the amplification remains

exponential. Once all ARTs hybridize to primer-probe, the amplification will be linear. The reaction will accumulate double stranded polynucleotides, single stranded PPS and PPi which can be labeled and detected.

6. The amplified products can be detected and quantified using any of the conventional detection systems for nucleic acids such as detection of fluorescent labels, enzyme-linked detection systems, microarray hybridization, capillary and gel electrophoresis, fluorescence polarization, mass spectrometry, Fluorescence Resonance Energy Transfer (FRET), Time-resolved fluorescence detection, electrical detection, and luminescence detection.

An example of a method of invention for genotyping SNPs is outlined below (FIG 8):

1 A target RNA or DNA sequence is allele-specifically hybridized to target complementary portions of amplification repeat templates (ART), while helper-primer anneals to helper-primer initiation portion of ART and to a region of target molecule which is adjacent to the hybridization region of ART's target complementary region.

2 A type IIS restriction enzyme (for example Fok I) nicks target DNA or RNA, because its opposite strand - ART contains modified nucleotides which render it resistant to cleavage. The nicking site is preferably also the SNP site.

3 The strand-displacing DNA polymerase extends the 3' end of nicked strand. Through subsequent repeat nicking and extension, multiple copies of PPS are generated, which can then anneal to free ARTs, prime new extension, nicking, and generation of new primer-probes.

4. The resulting amplified end products including double stranded DNA, single stranded PPS and PPI are then subjected to detection

An example of a method of invention for DNAzyme mediated detection of end products – single stranded PPS is outlined below (FIG. 10):

1. The template repeat portion of ART contains a complementary (antisense) sequence of a DNAzyme, for example 10-23 DNAzyme. During the chain reaction cascade of amplification, single-stranded PPS are produced that contain active (sense) copies of DNAzymes.
2. The DNA enzyme binds an RNA or DNA-RNA chimeric reporter substrate which contain fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site. Cleavage of this reporter substrate produces an increase in fluorescence that is indicative of successful amplification of the target initiated single stranded nucleic acid

I MATERIAL

A. Target Sequence

The target sequence, which is the object of hybridization to amplification template repeat (ART) and helper primer, and initiation of amplification and detection, can be any nucleic acid. The target sequence can be RNA, cDNA, genomic DNA, DNA contaminated by disease-causing microorganism and virus. The target sequence can also be DNA, RNA treated by chemical reagents, various enzymes and physical exposure.

B. Amplification repeat template (ART)

Amplification repeat template (ART) is a linear or circular single-stranded nucleic acid molecule, generally containing between 30 to 2000 nucleotides, preferably between about 40 to 300 nucleotides, and most preferably between about 50 to 150 nucleotides. Portions of ARTs have specific functions making the ART useful for combined exponential and linear amplification (CELA). These portions are referred to as the target complementary portion, template repeat portions, restriction enzyme site portion, RNA polymerase promoter portion, 3'end block portion and helper primer initiation portion. An ART may not contain all portions

An amplification repeat template (ART), when replicated, gives rise to multiple copies of a short DNA or RNA molecule containing sequence complementary to the template repeat portions with or without part of other portions. This short DNA or RNA molecule is referred to herein as primer-probe sequences (PPS).

1 Target complementary portion

There is generally one target complementary portion on ART, but more than one target complementary portions will also work. The target complementary portion can be any length that supports specific and stable hybridization between the target complementary portion and the target sequence. For this purpose, a length of 10 to 60 nucleotides for target complementary portion is preferred, with target probe portions 15 to 40 nucleotides long being most preferred.

The target complementary portion is complementary to a target nucleic acid sequence, such that upon hybridization a sequence element on the target complementary portion or a sequence element located on either sides of the target complementary portion initiates at least one nick on the target sequence under a specific enzymatic conditions. Such sequence elements having the nicking ability include but is not limited to restriction sites, RNase H digestion sites, other nuclease digestion sites. Any restriction enzyme sites can be used. For example, a type II restriction site is included within the target complementary portion (FIG. 1A and 1B), but the nucleotide(s) of digestion site on the target complementary portion of ART is modified so that the ART is resistant to digestion. Under restriction enzyme digestion condition, because of modified nucleotide(s) on the ART, the enzyme cuts the target sequence only

A type IIS restriction enzyme can also serve for this purpose The type IIS restriction enzyme recognition site does not need to be included within the target complementary portion, but can be on either sides of target complementary portion (FIG. 1D and 1E, FIG. 7A and 7B). Because the type IIS enzymes cut several bases away from

restriction recognition site, the digestion site will be on the target complementary portion. The nucleotide(s) on the digestion site of the target complementary portion is modified to block digestion of ART sequence but nick the target sequence. For SNP genotyping, it is preferred that the digestion site on target molecule is also the SNP site.

To be functional, the type IIS restriction site portion must be made double stranded for both its recognition and cleavage sites. There are two ways of making type IIS restriction site portion double stranded. First, a helper primer is annealed to type IIS restriction site portion only (FIG. 1D and 1E), or the 3' end of a helper primer anneals to type IIS restriction site portion and its 5' end anneals to a region of target molecule which is adjacent to the hybridization region of ART's target complementary region (FIG. 7A). Second, the 5'end of a helper primer anneals to target molecule region which is adjacent to the hybridization region of ART (FIG 7B), and its 3' end anneals to helper primer initiation portion of ART only. Upon extension of 3' end of helper primer on ART template by a DNA polymerase, a functional double stranded type IIS restriction site is created.

Alternatively, There are several nick enzymes available, for example, N.Bpu10I, N.BstSE. If a restriction site for one of these nicking enzymes is included in ART, no nucleotide modification in ART sequence is needed.

Any means for modifying nucleotide that support resistance of digestion can be used, for example phosphorothioate linkages between nucleotides, methylated nucleotides. The phosphorothioated nucleotides are preferred.

If the target sequence is RNA, upon hybridization to the target complementary portion, the target RNA sequence is nicked by nuclease, for example RNase H digestion at various non-specific sites. In one embodiment, part of the target complementary portion can be made by RNA (FIG. 3D). Upon hybridization between

target RNA sequence and the target complementary portion, the RNA/RNA hybrid will be resistant to digestion with RNase H.

2. Helper primer initiation portion

A helper primer initiation portion is included in ART when a helper primer is used in a reaction. Helper primer initiation portion is complementary to 3' end of helper primer, and is located between target complementary portion and type IIs restriction enzyme portion. Specifically the helper primer initiation portion is located between cleavage site of type IIS restriction enzyme in target complementary portion and recognition site of type IIS restriction enzyme. Its length is between 0 to 15 nucleotides, and 3 to 9 nucleotides are preferred.

3. Template repeat portions

There is at least one template repeat portion on ART, two is most preferred, and more than two will also work. If there is one template repeat portion (FIG. 1A), the ART will only support linear amplification. The template repeat portions are tandem repeated same sequences, which can be any length. The template repeat portions serve as template to generate multiple copies of amplification products – the primer-probe sequences. For this purpose, a length of 6 to 50 nucleotides for the template repeat portion is preferred, with template repeat portions 15 to 35 nucleotides long being most preferred The template repeat portions can have any desired sequence. In general, the sequence of the template repeat portions can be chosen such that it is nether significantly similar to any sequence in the mixture of target DNA or RNA, nor to any sequence of other ARTs in the CELA reaction.

In one embodiment, one of the template repeat portions can also be part of the target complementary portion (FIG 1G, FIG. 3C, 3D, FIG. 5B, 5C). In general, between two template repeat portions there is at lease one restriction enzyme site portion, or a RNA polymerase promoter portion The template repeat portion in the 3' end of ART

is referred to as upstream template repeat portion; the template repeat portion in 5' end of ART is referred to as downstream template repeat portion. The downstream template repeat portion is always located at the immediate 5'end of ART. The upstream template repeat portion can be located in front of target complementary portion (FIG. 1B, 1C, 1D, 1E), within target complementary portion (FIG. 1G) or after target complementary portion (FIG. 1H).

If DNAzyme is used for detecting single stranded primer probe sequences (PPS), the template repeat portion contains the catalytically inactive antisense sequence complementary to an active 10-23 DNAzyme or other DNAzyme (Santoro et al. Biochemstry 1998, 37, 13330-13342)

4 Restriction enzyme site portion

One type of restriction enzyme site portion is described in Section of target complementary portion. In general, the restriction enzyme site portion is a site supporting nicking opposite strand of an ART. Restriction enzyme site portions include modified nucleotides to make them resistant to nuclease digestion. For example, the restriction enzyme site portion can have one to ten phosphorothioate linkages (Taylor et al. Nucleic Acids Res, 1985, 13; 8749-64). Upon synthesis of complementary strand of ART, a restriction enzyme will nick the complementary strand of ART The restriction enzyme site portion can be located in any place of ART, but usually between the two template repeat portions. In some embodiments, the role played by restriction enzyme site portion can be replaced by RNA polymerase promoter portion (FIG. 6).

5. RNA polymerase promoter portion

As an alternative to restriction enzyme site portion, a RNA polymerase promoter portion can be included in an ART so that transcripts can be generated from template repeat portion sequence. RNA polymerase promoter portion comprise the sequence of

a promoter recognized by an RNA polymerase and a transcription initiation region which is located between the template repeat portion and the sequence of the promoter. The promoter may be the promoter for any suitable RNA polymerase. Examples of RNA polymerase are polymerases from E.coli and bacteriophages T7, T3 and SP6. Preferably the RNA polymerase is a bacteriophage-derived RNA polymerase, in particular the T7 polymerase. Because promoter sequences are generally recognized by specific RNA polymerases, the cognate polymerase for the promoter portion of the ART should be used for transcriptional amplification. The promoter portion can be located anywhere between the two template repeat portions. Preferably, the promoter portion is immediately adjacent to the downstream template repeat portion and is oriented to promote transcription toward the 5'end of the ART. This orientation results in transcripts that are complementary to template repeat portion sequence

6. 3' end block portion

It is preferred that ART is blocked by a blocking group at its 3' end, or is circular molecule (FIG. 1F) such that it is not extendible by a polymerase. The blockage of 3' end of ART can be achieved by any means known in the art. Blocking groups are chemical moieties which can be added to a nucleic acid to inhibit nucleic acid polymerization catalyzed by a nucleic acid polymerase. Blocking groups are typically located at the terminal 3' end of an ART which is made up of nucleotides or derivatives thereof. By attaching a blocking group to a terminal 3' OH, the 3' OH group is no longer available to accept a nucleoside triphosphate in a polymerization reaction.

Numerous different groups can be added to block the 3' end of a probe sequence. Examples of such groups include alkyl groups, non-nucleotide linkers, phosphorothioate, alkane-diol residues, peptide nucleic acid, and nucleotide derivatives lacking a 3' OH (e.g., cordycepin)

An alkyl blocking group is a saturated hydrocarbon up to 12 carbons in length which can be a straight chain or branched, and/or contain a cyclic group. More preferably, the alkyl blocking group is a C.sub 2 -C.sub.6 alkyl which can be a straight chain or branched, and/or contain a cyclic group.

7.Other moieties of ART

In certain embodiments, ART can include one or more moieties incorporated into 5' or 3' terminus or internally of primers that allow for the affinity separation of part of products associated with the label from unassociated part. Preferred capture moieties are those that can interact specifically with a cognate ligand For example, capture moiety can include biotin, digoxigenin etc. Other examples of capture groups include ligands, receptors, antibodies, haptens, enzymes, chemical groups recognizable by antibodies or aptamers. The capture moieties can be immobilized on any desired substrate. Examples of desired substrates include, e.g., particles, beads, magnetic beads, optically trapped beads, microtiterplates, glass slides, papers, test strips, gels, other mARTices, nitrocellulose, nylon. For example, when the capture moiety is biotin, the substrate can include streptavidin.

In one embodiment, ART molecules or a set of ART molecules are attached on a solid support, preferably the 3' end of ARTs are attached on a solid support. The solid support can include any solid material to which oligonucleotides can be coupled. This includes materials such as acrylamide, cellulose, nitrocellulose, glass, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfirmerate, collagen, glycosaminoglycans, and polyamino acids. Solid-state substrates can have any useful form including thin films or membranes, beads, bottles, dishes, fibers, woven fibers, shaped polymers, particles and microparticles. A preferred form for a solid-support is glass slides

Amplification repeat templates (ART) immobilized on a solid-state substrate allow capture of specific target molecules or amplification primer-probes on a solid-state detector. Such capture provides a convenient means for gene expression profiling and detecting multiple targets. For example, 3' ends of ARTs specific for multiple different target sequences can be immobilized on a glass slide, each in a different spot. Amplification of primer-probes specific for target sequences will occur only on those spots corresponding to ARTs for which the corresponding target sequences were present in a sample.

Methods for immobilization of oligonucleotides to solid-state substrates are well established. ART Oligonucleotides can be coupled to substrates using established coupling methods. For example, suitable attachment methods are described by Pease et al., Proc. Natl. Acad. Sci. USA 91(11):5022-5026 (1994), and Khrapko et al., Mol Biol (Mosk) (USSR) 25:718-730 (1991). A method for immobilization of 3'-amine oligonucleotides on casein-coated slides is described by Stimpson et al., Proc. Natl. Acad. Sci. USA 92:6379-6383 (1995). A preferred method of attaching oligonucleotides to solid-state substrates is described by Guo et al., Nucleic Acids Res. 22:5456-5465 (1994).

C Helper primer

Helper primer is an oligonucleotide that helps to make double stranded functional type IIS restriction enzyme site in ART. It can be any length, as long as it efficiently hybridizes to restriction site portion of ART, or efficiently hybridizes to target molecule or efficiently hybridizes to target molecule and helper primer initiation portion of ART. Helper primer can contain any desired nucleotide modifications.

D. Detection labels

To aid in detection and quantitation of nucleic acids amplified using CELA, detection labels can be directly incorporated into amplified nucleic acids or can be coupled to

detection molecules. As used herein, a detection label is any molecule that can be associated with amplified nucleic acid, directly or indirectly, and which results in a measurable, detectable signal, either directly or indirectly. Many such labels for incorporation into nucleic acids or coupling to nucleic acid or antibody probes are known to those of skill in the art. Examples of detection labels suitable for use in CELA are radioactive isotopes, fluorescent molecules, phosphorescent molecules, enzymes, antibodies, and ligands.

Examples of suitable fluorescent labels include but are not limited to fluorescein (FITC), 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, 4'-6-diamidino-2-phenylinodole (DAPI), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. Preferred fluorescent labels are fluorescein (5-carboxyfluorescein-N-hydroxysuccinimide ester) and rhodamine (5,6-tetramethyl rhodamine) Preferred fluorescent labels for combinatorial multicolor coding are FITC and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. The absorption and emission maxima, respectively, for these fluors are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm), thus allowing their simultaneous detection. The fluorescent labels can be obtained from a variety of commercial sources, including Molecular Probes, Eugene, Oreg. and Research Organics, Cleveland, Ohio

Labeled nucleotides are a preferred form of detection label since they can be directly incorporated into the products of CELA during synthesis. Examples of detection labels that can be incorporated into amplified DNA or RNA include nucleotide analogs such as BrdUrd (Hoy and Schimke, Mutation Research 290:217-230 (1993)), BrUTP (Wansick et al., J. Cell Biology 122:283-293 (1993)) and nucleotides modified with biotin (Langer et al., Proc. Natl. Acad. Sci. USA 78:6633 (1981)) or with suitable haptens such as digoxygenin (Kerkhof, Anal. Biochem. 205:359-364 (1992)). Suitable fluorescence-labeled nucleotides are Fluorescein-isothiocyanate-dUTP, Cyanine-3-dUTP and Cyanine-5-dUTP (Yu et al., Nucleic Acids Res.,

22:3226-3232 (1994)) A preferred nucleotide analog detection label for DNA is BrdUrd (BUDR triphosphate, Sigma), and a preferred nucleotide analog detection label for RNA is Biotin-16-uridine-5'-triphosphate (Biotin-16-dUTP, Boebringher Mannheim). Fluorescein, Cy3, and Cy5 can be linked to dUTP for direct labeling. Cy3.5 and Cy7 are available as avidin or anti-digoxygenin conjugates for secondary detection of biotin- or digoxygenin labeled probes.

Detection labels that are incorporated into amplified nucleic acid, such as biotin, can be subsequently detected using sensitive methods well-known in the art. For example, biotin can be detected using streptavidin-alkaline phosphatase conjugate (Tropix, Inc.), which is bound to the biotin and subsequently detected by chemiluminescence of suitable substrates (for example, chemiluminescent substrate CSPD: disodium, 3-(4methoxyspiro-[1,2,-dioxetane-3-2'-(5'-chloro)tricyclo [3.3.1.1.sup.3,7]decane]-4-yl) phenyl phosphate; Tropix, Inc.).

A preferred detection label for use in detection of amplified RNA is acridinium-ester-labeled DNA probe (GenProbe, Inc., as described by Arnold et al., Clinical Chemistry 35:1588-1594 (1989)) An acridinium-ester-labeled detection probe permits the detection of amplified RNA without washing because unhybridized probe can be destroyed with alkali (Arnold et al. (1989)).

Molecules that combine two or more of these detection labels are also considered detection labels. Any of the known detection labels can be used with the disclosed probes, tags, and method to label and detect nucleic acid amplified using the disclosed method. Methods for detecting and measuring signals generated by detection labels are also known to those of skill in the art. For example, radioactive isotopes can be detected by scintillation counting or direct visualization; fluorescent molecules can be detected with fluorescent spectrophotometers; phosphorescent molecules can be detected with a spectrophotometer or directly visualized with a camera, enzymes can be detected by detection or visualization of the product of a reaction catalyzed by the enzyme; antibodies can be detected by detecting a

secondary detection label coupled to the antibody. Such methods can be used directly in the disclosed method of amplification and detection As used herein, detection molecules are molecules which interact with amplified nucleic acid and to which one or more detection labels are coupled.

E. Reporter substrate

A reporter substrate molecule can be an RNA or DNA-RNA chimera which contain fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site (Santoro et al. Biochemstry 1998, 37, 13330-13342, Todd, et al. Clinical Chemistry, 2000, 46:5, 625-630). Any fluorophores and any quenchers can be incorporated at any desired places into a reporter substrate. One example is that the reporter 6-carboxyfluorescein (RAM) is incorporated at the 5' end, and the quencher 6-carboxytetramethylrhodamine (TAMRA) or 4-(4-dimethylaminophenylazo)benzoic acid (DABCYL) is incorporated internally. Any blocking moiety such as 3' phosphate group can be added to the 3' end to prevent extension by DNA polymerase during reaction. Cleavage of this reporter substrate produces an increase in fluorescence that is indicative of successful amplification of the target gene or transcript.

D. DNA polymerases

For combined exponential and linear amplification (CELA), it is preferred that a DNA polymerase be capable of displacing the strand complementary to the template strand, termed strand displacement, and lack a 5' to 3' exonuclease activity. Strand displacement is necessary to result in synthesis of multiple copies of the PPS. A 5' to 3' exonuclease activity, if present, might result in the destruction of the synthesized strand. It is also preferred that DNA polymerases for use in the disclosed method are highly processive Preferred DNA polymerases are bacteriophage .phi 29 DNA polymerase (U.S.Pat. Nos. 5,198,543 and 5,001,050 to Blanco et al.), phage M2 DNA polymerase (Matsumoto et al., Gene 84.247 (1989)), phage .phi.PRD1 DNA polymerase (Jung et al., Proc. Natl. Acad.

Sci. USA 84·8287 (1987)), VENT.RTM. DNA polymerase (Kong et al., J. Biol. Chem 268:1965-1975 (1993)), Klenow fragment of DNA polymerase I (Jacobsen et al., Eur. J Biochem. 45.623-627 (1974)), T5 DNA polymerase (Chatterjee et al., Gene 97:13-19 (1991)), PRD1 DNA polymerase (Zhu and Ito, Biochim. Biophys. Acta 1219·267-276 (1994)), modified T7 DNA polymerase (Tabor and Richardson, J Biol. Chem. 262:15330-15333 (1987); Tabor and Richardson, J. Biol. Chem. 264·6447-6458 (1989); Sequenase.TM. (U S. Biochemicals)), T4 DNA polymerase holoenzyme (Kaboord and Benkovic, Curr. Biol. 5·149-157(1995)), Bca polymerase (Takara) and Bst polymerase (NEB). .phi 29 and Bst DNA polymerases are most preferred

Strand displacement can be facilitated through the use of a strand displacement factor, such as helicase. It is considered that any DNA polymerase that can perform Strand displacement in the presence of a strand displacement factor is suitable for use in the disclosed method, even if the DNA polymerase does not perform Strand displacement in the absence of such a factor. Strand displacement factors useful in CELA include BMRF1 polymerase accessory subunit (Tsurumi et al., J. Virology 67(12):7648-7653 (1993)), adenovirus DNA-binding protein (Zijderveld and van der Vliet, J. Virology 68(2):1158-1164 (1994)), herpes simplex viral protein ICP8 (Boehmer and Lehman, J. Virology 67(2).711-715 (1993); Skaliter and Lehman, Proc. Natl. Acad. Sci. USA 91(22):10665-10669 (1994)), single-stranded DNA binding proteins (SSB; Rigler and Romano, J. Biol. Chem. 270·8910-8919 (1995)), and calf thymus helicase (Siegel et al., J. Biol. Chem. 267:13629-13635 (1992)).

E. RNA polymerases

Any RNA polymerase which can carry out transcription in vitro and for which promoter sequences have been identified can be used in the disclosed CELA method. Stable RNA polymerases without complex requirements are preferred. Most preferred are T7 RNA polymerase (Davanloo et al., Proc. Natl. Acad. Sci USA 81:2035-2039 (1984)) and SP6 RNA polymerase (Butler and Chamberlin, J. Biol. Chem. 257:5772-5778 (1982)) which are highly specific for particular promoter sequences (Schenborn and Meirendorf,

Nucleic Acids Research 13:6223-6236 (1985)). Other RNA polymerases with this characteristic are also preferred Because promoter sequences are generally recognized by specific RNA polymerases, the ART should contain a promoter sequence recognized by the RNA polymerase that is used Numerous promoter sequences are known and any suitable RNA polymerase having an identified promoter sequence can be used. Promoter sequences for RNA polymerases can be identified using established techniques.

F. Restriction endonuclease and ribonuclease

The disclosed method makes use of restriction enzymes (also referred to as restriction endonucleases) for cleaving one strand of double stranded nucleic acids. Other nucleic acid cleaving reagents also can be used. Preferred nucleic acid cleaving reagents are those that cleave nucleic acid molecules in a sequence-specific manner. Many restriction enzymes are known and can be used with the disclosed method. Restriction enzymes generally have a recognition sequence and a cleavage site. It is preferred that restriction enzymes are chosen so that the enzyme nick unmodified DNA strand only.

In one embodiment, nicking the hybridized target RNA at predetermined RNA sequences is carried out with a double-stranded ribonuclease. Such ribonucleases nick or excise ribonucleic acid sequences from double-stranded RNA/DNA hybridized strands. An example of a ribonuclease useful in the practice of this invention is RNase H. RNase H is a RNA specific digestion enzyme which cleaves RNA found in DNA/RNA hybrids in a non-sequence-specific manner. Other ribonucleases and enzymes may be suitable to nick or excise RNA from RNA/DNA strands, such as Exo III and reverse transcriptase.

The materials described above can be packaged together in any suitable combination as a kit useful for performing the disclosed method.

II. Method

The disclosed combined exponential and linear amplification (CELA) methods involve nicking target sequence by a nuclease, extension from nicking site by a strand-displacing DNA polymerase, nicking and extension again to generate multiple copies of single stranded PPS. The single stranded PPS serves as primer to anneal to free ARTs and prime extension and nicking. Exponential amplification takes place during repeated replication of PPS which anneal to new ARTs and prime new primer-probe generation. Linear amplification takes place when all ART molecules hybridize to primer-probes and become double stranded.

In one embodiment of the invention (FIG. 2), the combined exponential and linear amplification (CELA) of PPS depends on the hybridization between target DNA or RNA sequence and target complementary portion of amplification repeat templates (ART) In the presence of a DNA or RNA molecule having the target sequence, target complementary portion of ART can hybridize to the target sequence allowing the target sequence to be nicked by a restriction enzyme. By strand-displacing polymerase extension of nicked strand, and subsequent repeat nicking and extension, multiple copies of PPS are generated which can then anneal to free ARTs, prime new extension, and generate new PPS Alternatively, if a RNA polymerase promoter portion is present in the ART (FIG. 6), the reaction with a RNA polymerase and appropriate condition generates multiple copies of PPS RNA transcripts, which can anneal to free ARTs, prime new extension, and generate new PPS This form of method is useful, for example, for determining which target sequences are present in a sample, or for determining which samples contain a target sequence, or how much target sequence is present in a sample. It is also useful for detecting mutations, genotyping SNP

In another embodiment of the invention (FIG. 4), the amplification of PPS depends on the hybridization of target RNA sequence and target complementary portion of ART. In the presence of a RNA molecule having the target sequence, the target complementary portion of ART can hybridize to the target sequence allowing the target RNA sequence to

be nicked by ribonuclease, for example RNase H. By strand-displacing polymerase extension of the nicked RNA strand, and subsequent repeat nicking and extension, multiple copies of PPS are generated which can then anneal to free ARTs, prime new extension, and generate new PPS Alternatively, if a RNA polymerase promoter portion is present in the ART (FIG. 6), the reaction with a RNA polymerase and appropriate condition generates multiple copies of PPS RNA transcripts, which can anneal to free ARTs, prime new extension, and generate new PPS. This form of method is useful, for example, for determining which target RNA sequences are present in a sample, or for determining which samples contain a target RNA sequence, or how much target sequence is present in a sample. It is extremely useful for quantifying virus RNA load in patient sample. It is also useful for mRNA profiling, where other approach may not be sensitive enough to detect changes in the concentration of low abundance gene products, or in a little amount of starting material, such as a single cell.

In another embodiment of the invention, CELA can be used for genotyping SNPs. Two types of ARTs are included in a SNP genotyping reaction: first ART contains an allele-specific target complementary portion, second ART contains another allele-specific target complementary portion The template repeat portions of two ARTs are also different such that their corresponding PPS can be distinguished by a detection method. A preferred detection method is DNAzyme mediated detection which is disclosed in this invention. A target RNA or DNA sequence is allele-specifically hybridized to target complementary portion of amplification repeat templates (ARTs), while a helper-primer anneals to helper-primer initiation portion of ART, and anneals to a region of target molecule which is adjacent to the hybridization region of ART's target complementary region. A DNA polymerase extends 3' end of helper-primer on template repeat portion of ART to create double stranded functional type IIS restriction site A type IIS restriction enzyme (for example Fok I) nicks target DNA or RNA in hybrid DNA/DNA or RNA.DNA (Kim, et al., Science, 1988, 240:504-6), because its opposite strand - ART contains modified nucleotides which render it resistant to cleavage. The strand-displacing DNA polymerase extends the nicked strand Through subsequent repeat nicking and extension, multiple copies of PPS are generated, which can then anneal to free ARTs, prime new extension,

nicking, and generation of new PPS. The resulting amplified end products including double stranded DNA, single stranded PPS and PPI are then subjected to detection

In a further embodiment, the invention makes use of DNAzyme for detection of end products – single stranded PPS The template repeat portion of ART contains a complementary (antisense) sequence of a DNAzyme, for example 10-23 DNAzyme (Santoro et al. Biochemstry 1998, 37, 13330-13342, Todd, et al. Clinical Chemistry, 2000, 46·5, 625-630). During the chain reaction cascade of amplification, single-stranded PPS are produced that contain active (sense) copies of DNAzymes. The DNA enzyme binds an RNA or DNA-RNA chimeric reporter substrate which contain fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site. Cleavage of this reporter substrate produces an increase in fluorescence that is indicative of successful amplification of the target initiated single stranded PPS.

A. Target specific hybridization or allele specific hybridization

A amplification repeat templates (ART) or a set of ARTs or a mixture of ARTs and helper primer are incubated with a sample containing DNA, RNA, or both, under suitable hybridization conditions, so that a double stranded DNA/DNA or RNA/DNA in the target complementary portions of ART or helper primers are formed A stringent hybridization condition allows subsequent amplification to be dependent on the perfect match between a target sequence and ART so that allele discrimination can be achieved.

B Forming functional restriction site

If a restriction enzyme recognition site is located on the target complementary portion of ART (FIG. 1A, B, C, F, G, H), a double stranded functional restriction site is already formed after target specific hybridization between target molecule and ART (FIG. 2). In another embodiment, a nick restriction enzyme is used, which usually cut one strand only (FIG 1C). In this case, a modified nucleotide in the ART is not required

In the case of that target molecule is RNA and restriction site is located outside of target complementary portion of ART, a functional double stranded restriction site is created by nicking target RNA strand by a nuclease, for example RNase H, digestion and extension of 3' end of nicked strand on ART template by a strand displacing DNA polymerase (FIG. 3 and FIG. 4). RNase H is a RNA specific digestion enzyme which cleaves RNA found in DNA/RNA hybrids in a non-sequence-specific manner To prevent complete digestion away of RNA strand, a portion of target complementary portion of ART is made by RNA (FIG. 3D), thus RNA/RNA hybrid is resistant to be digested by RNase H.

For genotyping, allele-specific ARTs, helper primer and type IIS restriction enzyme are included in a reaction (FIG 7 and FIG. 8) The advantage of using type IIs restriction enzyme is that a target sequence for analyzing does not need to be restricted to contain a restriction enzyme site. A universal restriction enzyme can be used in all detections. The type IIS restriction enzyme site is usually located outside on either sides of target complementary portion of ART (FIG. 1D and 1E, 7A and 7B) One of type IIS restriction enzymes usually used in this reaction is Fok I. Fok I restriction enzyme can cleave DNA at any predetermined site with oligodeoxynucleotide adapter-primers. The adapter-primer in this invention is ART. The target complementary portion of ART contains Fok I digestion site (also preferably the SNP site), and the downstream of the target complementary portion of ART contains Fok I recognition site. The target complementary portion of ART selects a complementary sequence on the target denatured DNA or RNA, hybridizes with it to form the double stranded cleavage site, and serves as a primer to convert the ART to double stranded DNA (Kim et al., Science, 1988, 240, 504-6). However before Fok I enzyme can cleave (nick) target strand, the single strand Fok I recognition site on ART must be converted to functional double stranded DNA. This is accomplished by helper primer One of preferred forms of helper primer is shown in FIG 7B and FIG 8 The helper primer and ART each includes a target complementary portion that can hybridize to the target at adjacent sites and another much short portion (3-9 nucleotides) that hybridize to each other. The helper primer and ART are designed such that they can only anneal to each other in the presence of the specific target. Following helper primer anneals to ART in the presence of a target sequence, a

strand displacing DNA polymerase extends the 3' end of helper primer by copying template repeat portion of ART to produce a double stranded functional Fok I site (FIG. 8)

C Nicking

Once double stranded functional restriction site is formed, its cognate restriction enzyme acts on the double stranded nucleic acid, and nicks the opposing strand of ART

In another embodiment of invention, the target complementary portion of ART hybridizes to a free 3' end of target DNA or RNA molecule. In this situation, nicking the target sequence by an enzyme used in all other embodiments is not required.

For genotyping, high specificity is achieved by three factors. First, allele-specific hybridization of target complementary portions of two allele-specific ARTs to perfect match target sequences gives certain specificity. Second, the helper primer can anneal to ART and be extended to create functional Fok I site only in the presence of target sequence Third, if occasionally a non allele-specific hybridization occur, the Fok I enzyme will not cleave or very inefficiently cleave a mismatch at digestion site (here also SNP site) so that a chain reaction will not happen.

D. Extension of the nicked strand

Once the opposite strand of ART is nicked, a DNA polymerase catalyzes nicked 3' end extension and strand displacement on the template of ART. The nicked 3' end extension on the ART goes beyond at least one unit of template repeat portions, optional another restriction enzyme site portion or RNA polymerase promoter portion

E Repeat nicking and extension to generate multiple copies of single stranded PPS

The newly synthesized strand from step D is nicked again either at the same site used in the original nicking with the same restriction enzyme, or at another restriction site

downstream of target complementary portion. In one embodiment, the original nicking is in the RNA strand of RNA/DNA hybrid, the newly synthesized strand from step D is nicked at a restriction site downstream of target complementary portion In another embodiment, a RNA polymerase promoter portion is present downstream of target complementary portion, in this situation a restriction enzyme site and nicking of newly synthesized strand are not required.

A DNA polymerase acts on the nicked strand, catalyzes extension and strand displacement on the template of ART. The processes of nicking and extension are repeated many times to generate multiple copies of single stranded primer-probe sequences (PPS). In one embodiment, a RNA polymerase promoter portion is present downstream of target complementary portion. Once the promoter portion becomes double stranded, the RNA polymerase acts on the promoter and generates multiple copies of RNA transcripts, i.e. single stranded primer-probe RNA sequences.

F Annealing and extension of PPS on free amplification repeat templates (ARTs), triggering chain reaction cascade

The single stranded primer-probes generated from above step act as primers to anneal to upstream unit of free template repeat portions of ART and are extended by a DNA polymerase. These extended sequences are nicked and extended again to generate multiple copies of PPS. The new PPS will find another free ART, and cause a chain reaction cascade. In one embodiment, if the ART contains a RNA polymerase promoter sequence, multiple copies of single stranded RNA PPS will be generated from the template repeat portion of ART.

Because all reagents are in single tube, all above steps will occur simultaneously, there is no distinct boundary between the steps. The step E and F are repeated many times. As long as there is free ART (which is not met with a primer-probe), the amplification remains exponential. Once all ARTs are hybridized to PPS, the amplification will be

linear The reaction accumulates double stranded polynucleotides, single stranded PPS and PPi which can be labeled and detected.

G. Detection

The amplified double stranded ARTs, single stranded PPS and PPi can be detected and quantified using any of the conventional detection systems. For examples, the double stranded ARTs and single stranded PPS can be detected by Fluorescence Detection, Fluorescence Polarization, Fluorescence Resonance Energy Transfer, Mass Spectrometry, Electrical Detection, and Microarray. Specifically, the double stranded ARTs can be detected by binding to a double strand specific fluorescence dye SYBR green. In this invention, the single stranded PPS is detected by DNAzyme mediated cleavage as outlined below. The PPi generated can be converted to ATP and the resulting ATP concentration is detected and quantified with firefly luciferase (FIG. 9).

CELA products may be detected by incorporating labeled moieties, such as fluorescent nucleotides, biotinylated nucleotides, digoxygenin-containing nucleotides, or bromodeoxyuridine,

In one embodiment for gene expression profiling, a microarray detection system can be used. CELA is multiplexed by using a set of different amplification repeat templates (ARTs), each ART carrying different target complementary portions designed for binding to specific target genes, and each ART also carrying different template repeat portions designed for primer-probe binding to specific oligonucleotides on the microarray Only those ARTs that are able to find their targets will give rise to specific PPS In another embodiment for gene expression profiling, ARTs are spotted on microarray. During CELA reaction, double stranded ARTs are created and can be detected and quantified using various detection systems. One of detection system is using SYBR green staining that can be monitored in real time.

H. DNAzyme mediated detection of end products - single stranded PPS

The invention also makes use of DNAzyme for detection of end products – single stranded PPS (FIG. 10). The template repeat portions of ART contain a complementary (antisense) sequence of a DNAzyme, for example 10-23 DNAzyme (Santoro et al. Biochemstry 1998, 37, 13330-13342, Todd, et al. Clinical Chemistry, 2000, 46:5, 625-630). During the chain reaction cascade of amplification, single-stranded PPS are produced that contain active (sense) copies of DNAzymes. The DNA enzyme binds an RNA or DNA-RNA chimeric reporter substrate which contain fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site. Cleavage of this reporter substrate produces an increase in fluorescence that is indicative of successful amplification of the target initiated single stranded PPS.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are as described. All publication cited herein are hereby incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein.

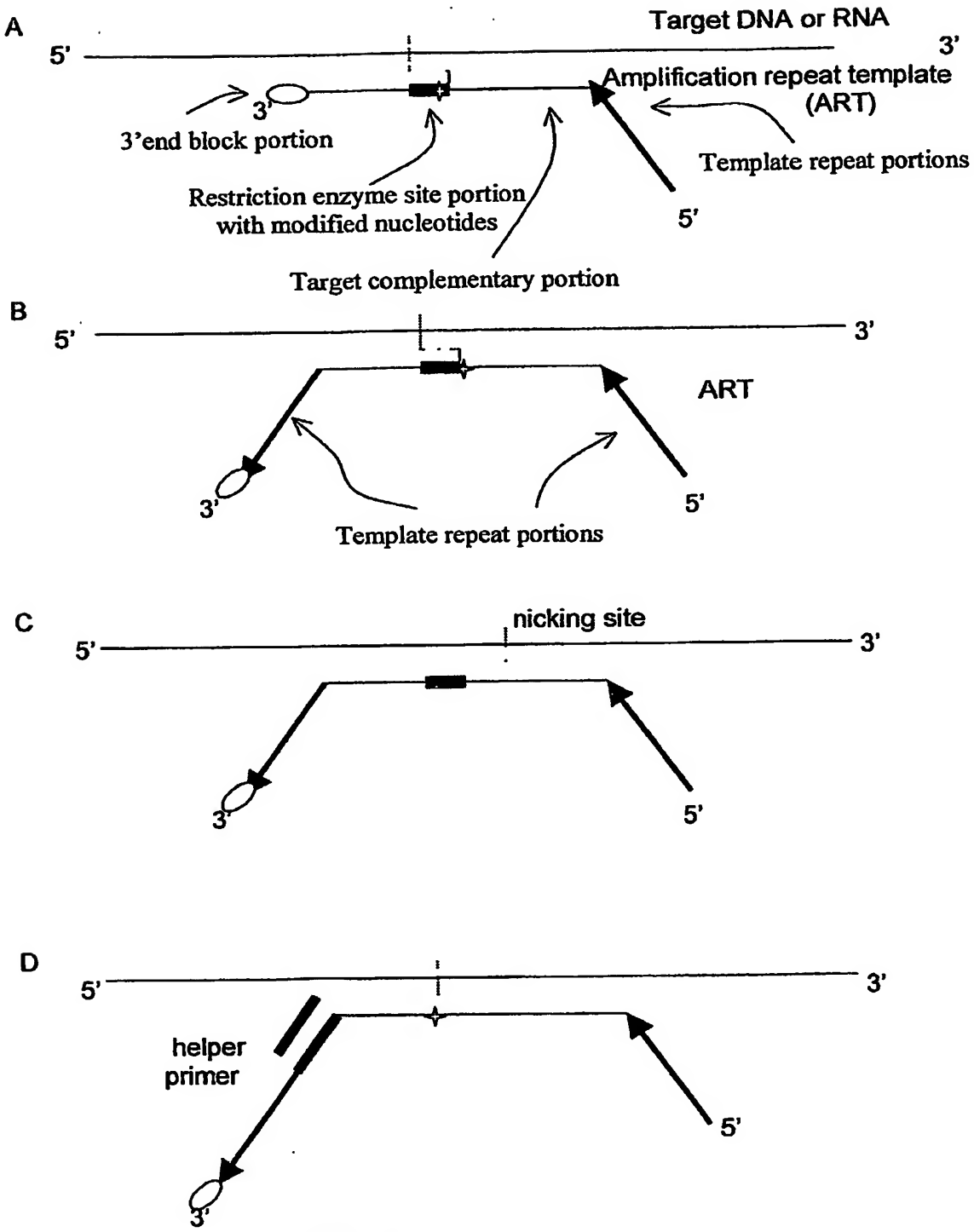

A
Target DNA or RNA
5'
3'
Amplification repeat template
(ART)
3'
3'end block portion
Template repeat portions
Restriction enzyme site portion
with modified nucleotides
5'
Target complementary portion
B
5'
3'
ART
3'
5'
Template repeat portions
C
nicking site
5'
3'
3'
5'
D
5'
3'
helper
primer
5'
3'

FIG. 1

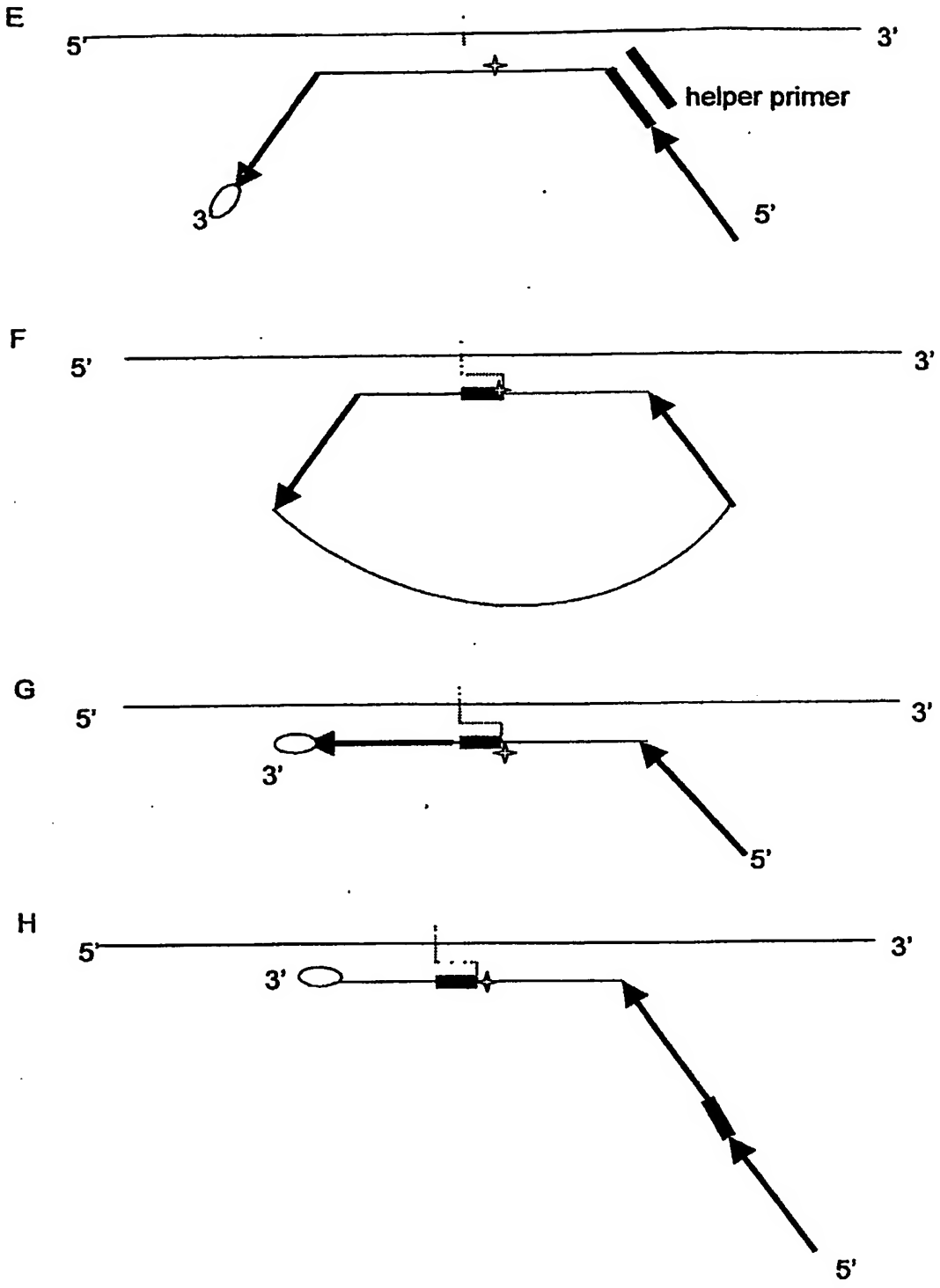


FIG. 1

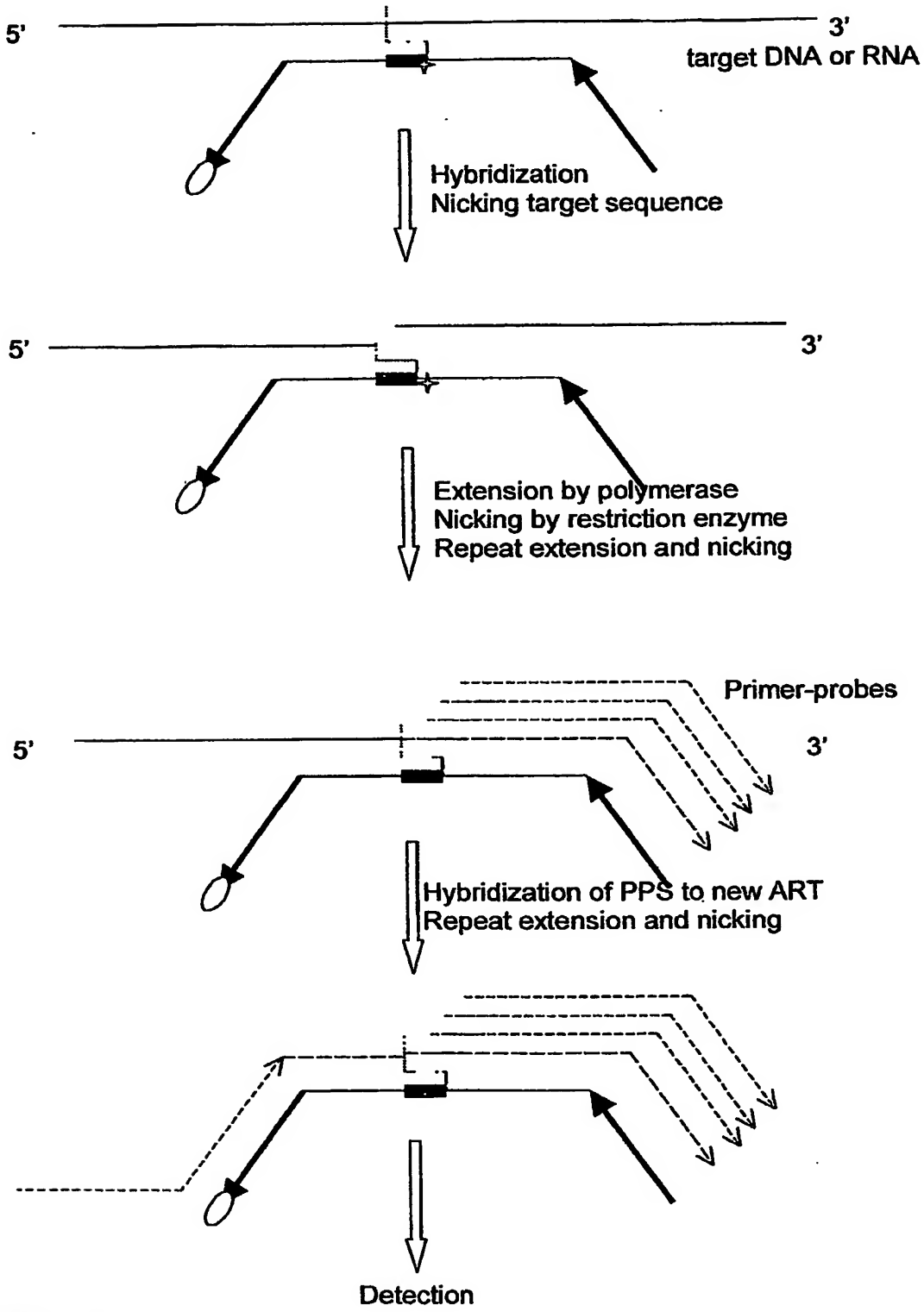

5'
3'
target DNA or RNA
Hybridization
Nicking target sequence
5'
3'
Extension by polymerase
Nicking by restriction enzyme
Repeat extension and nicking
Primer-probes
5'
3'
Hybridization of PPS to new ART
Repeat extension and nicking
Detection

FIG. 2

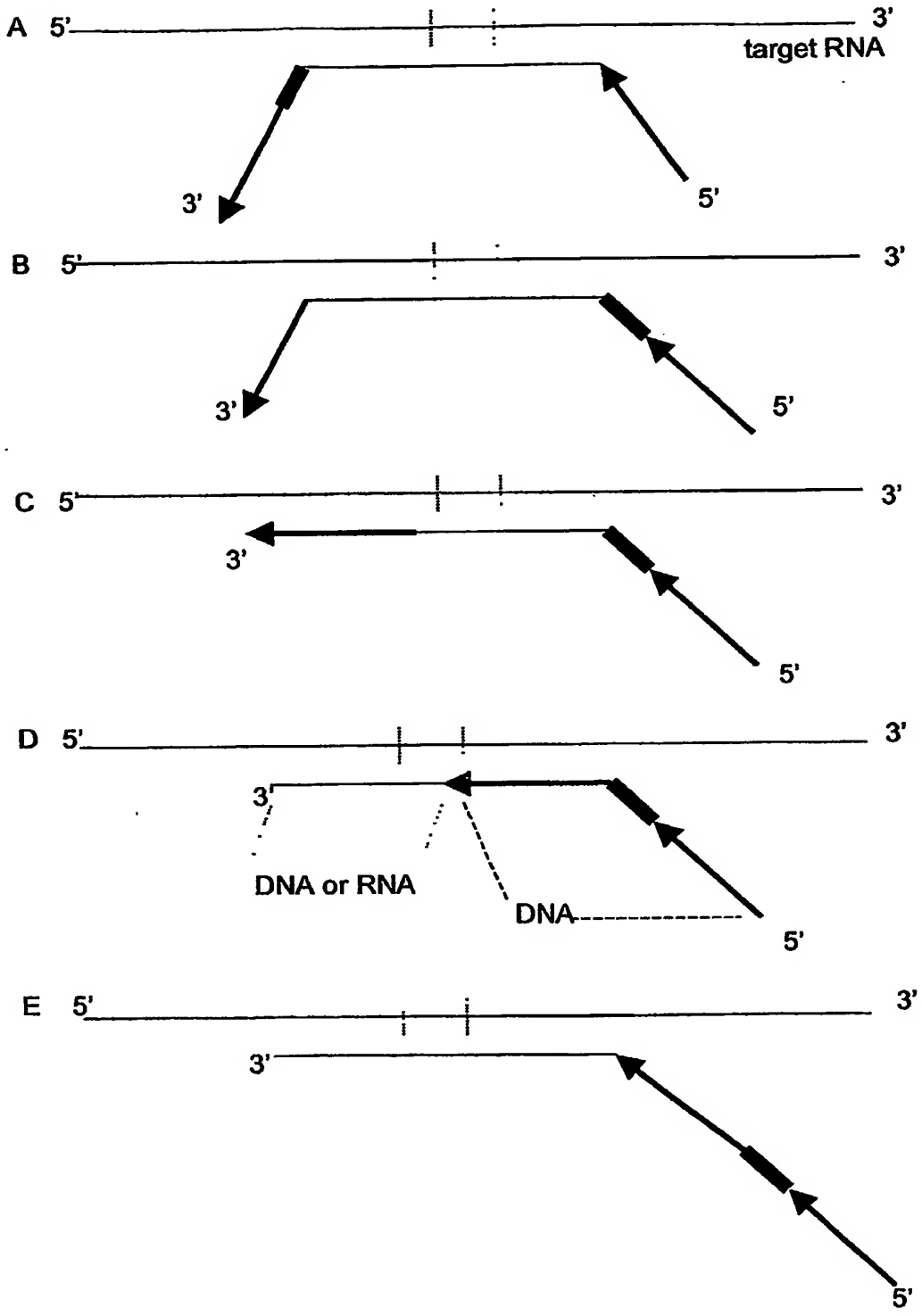


FIG. 3

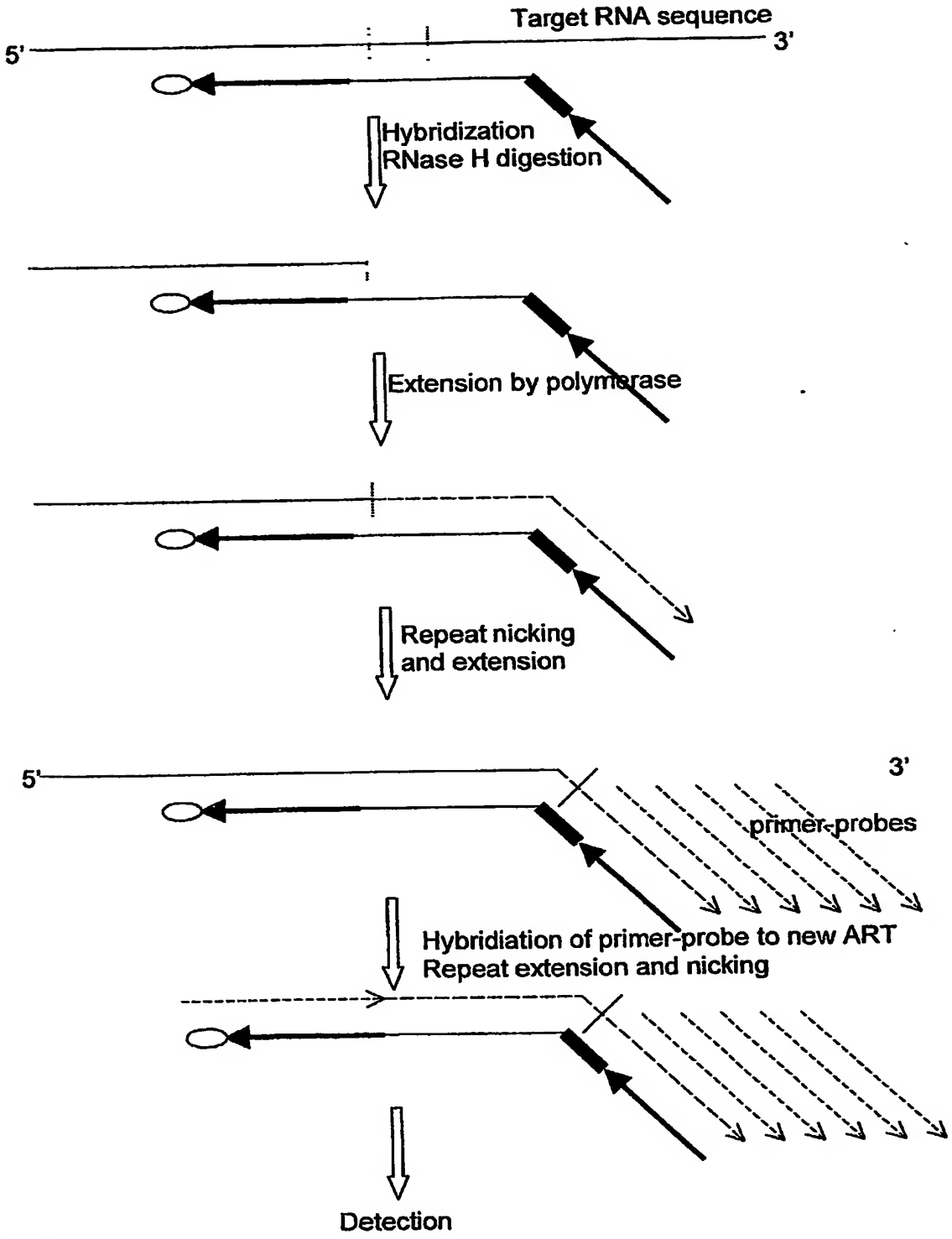

Target RNA sequence
5'
3'
Hybridization
RNase H digestion
Extension by polymerase
Repeat nicking
and extension
5'
3'
primer-probes
Hybridiation of primer-probe to new ART
Repeat extension and nicking
Detection

FIG. 4

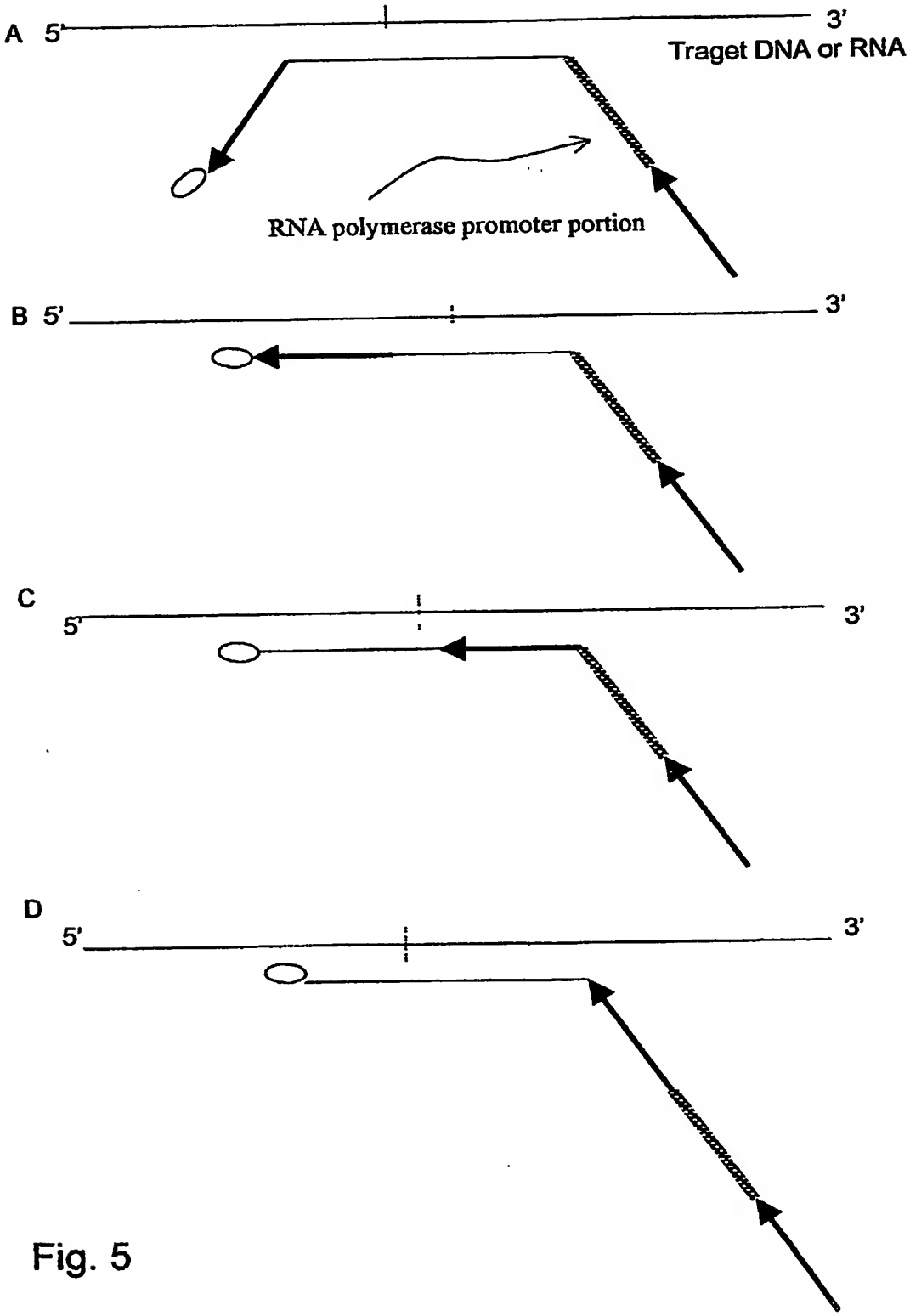

A 5'
3'
Traget DNA or RNA
RNA polymerase promoter portion
B 5'
3'
C
5'
3'
D
5'
3'

Fig. 5

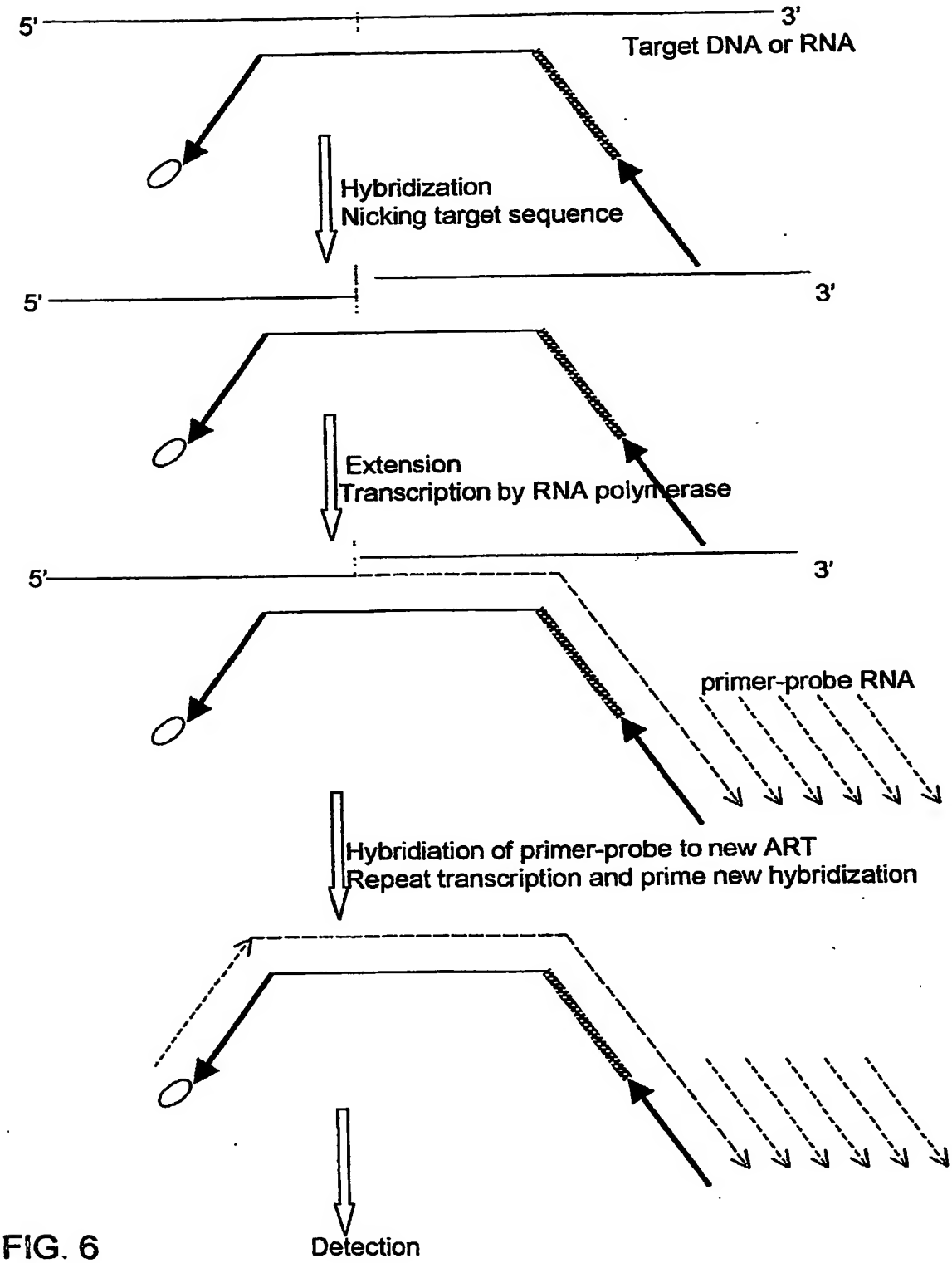

5'
3'
Target DNA or RNA
Hybridization
Nicking target sequence
5'
3'
Extension
Transcription by RNA polymerase
5'
3'
primer-probe RNA
Hybridiation of primer-probe to new ART
Repeat transcription and prime new hybridization
Detection

FIG. 6

Examples of helper primer and ART with type IIS restriction site

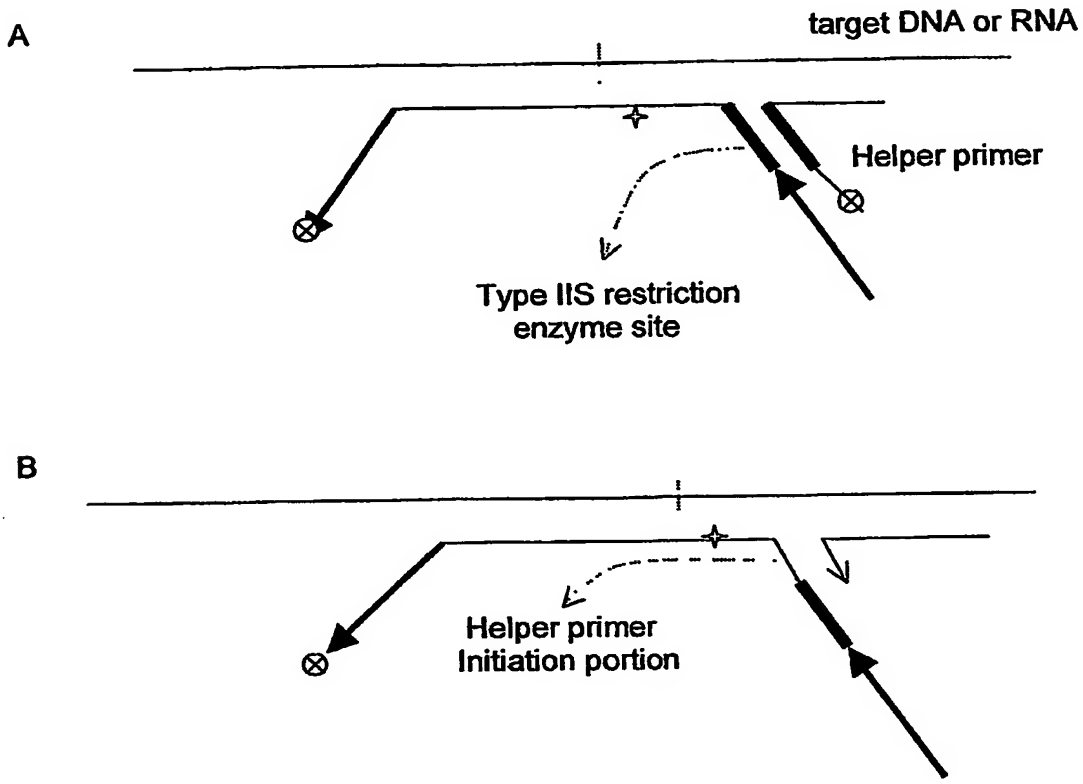


FIG. 7

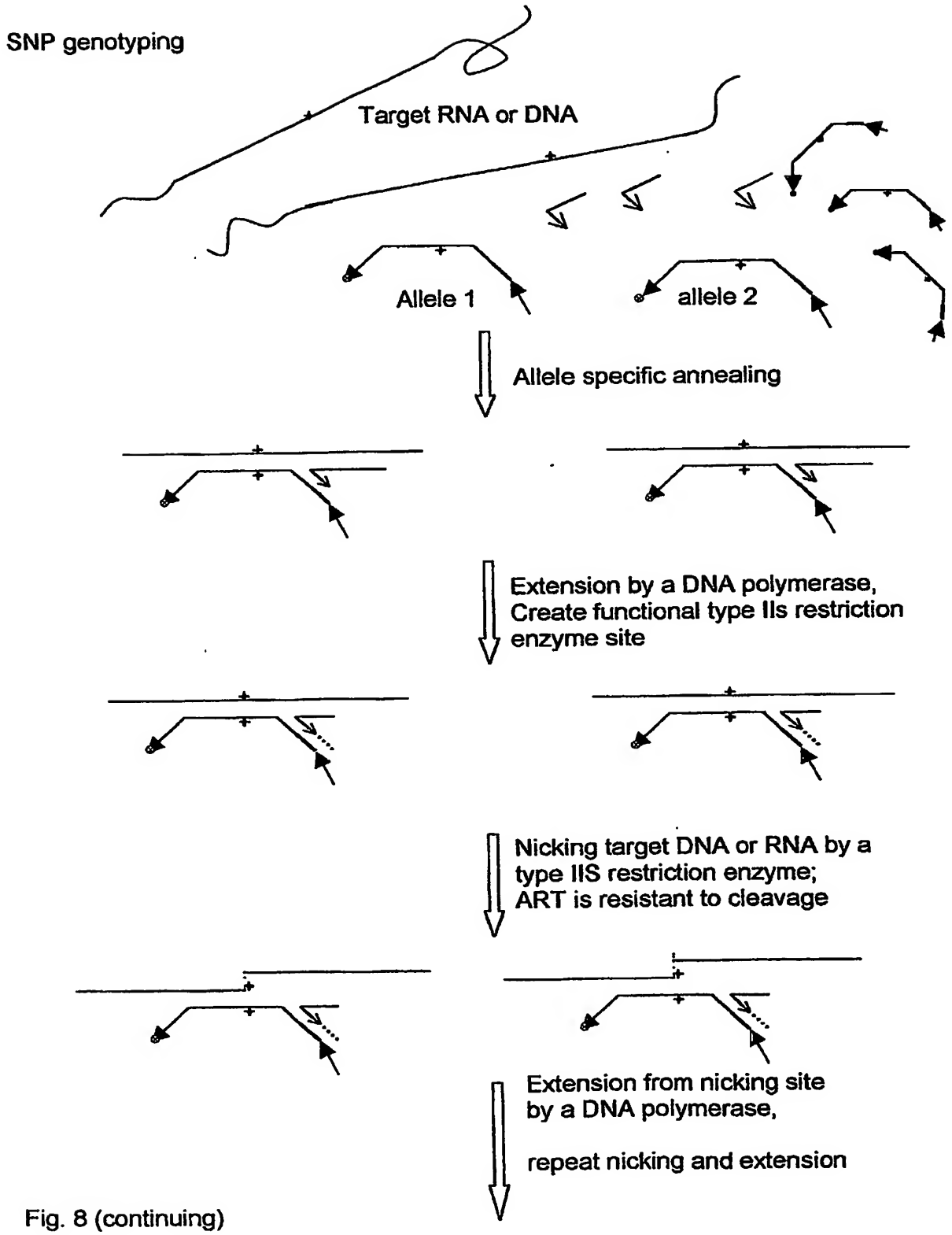
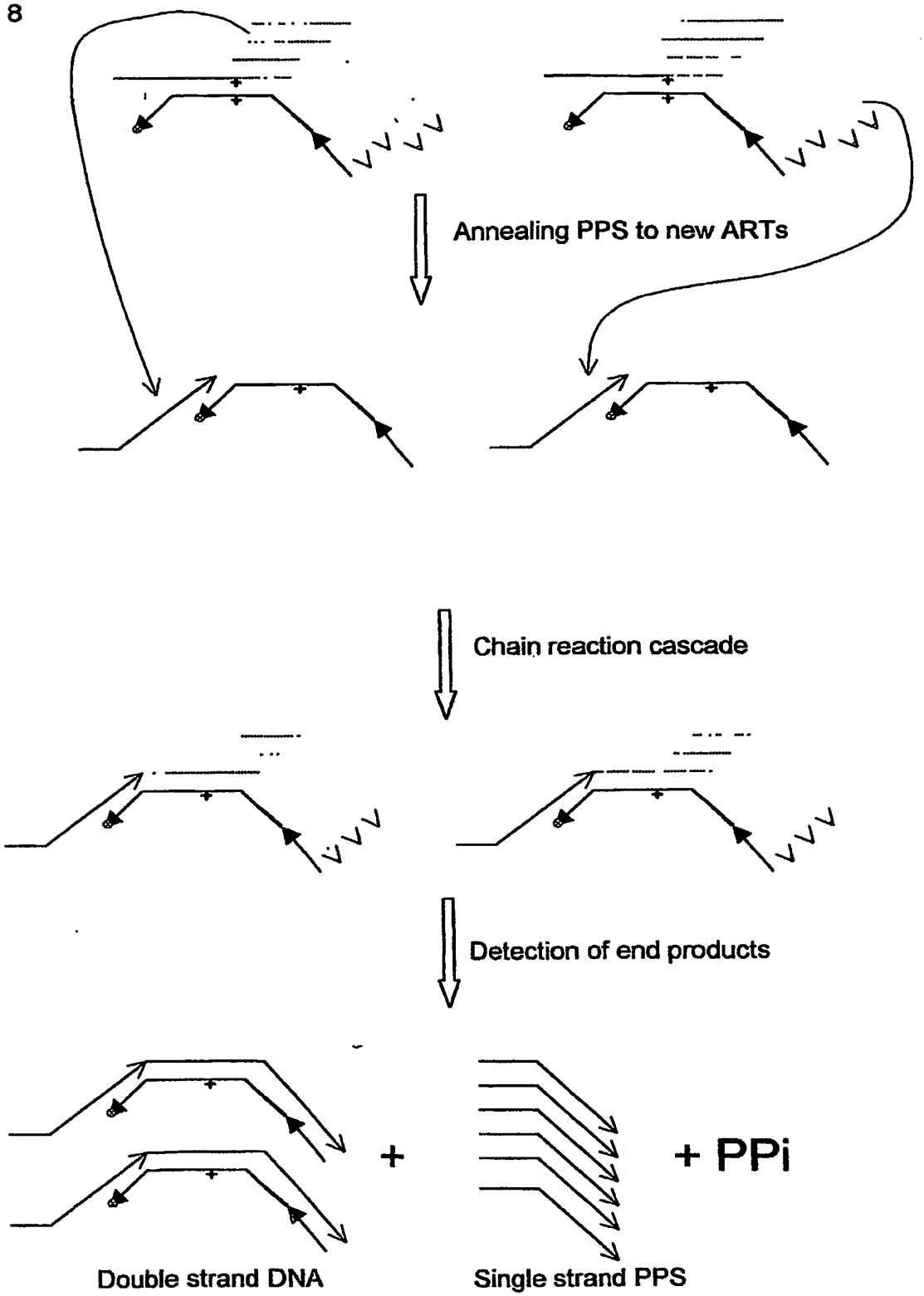


Fig. 8 (continuing)

Fig. 8


Annealing PPS to new ARTs
Chain reaction cascade
Detection of end products
+
+ PPi
Double strand DNA
Single strand PPS

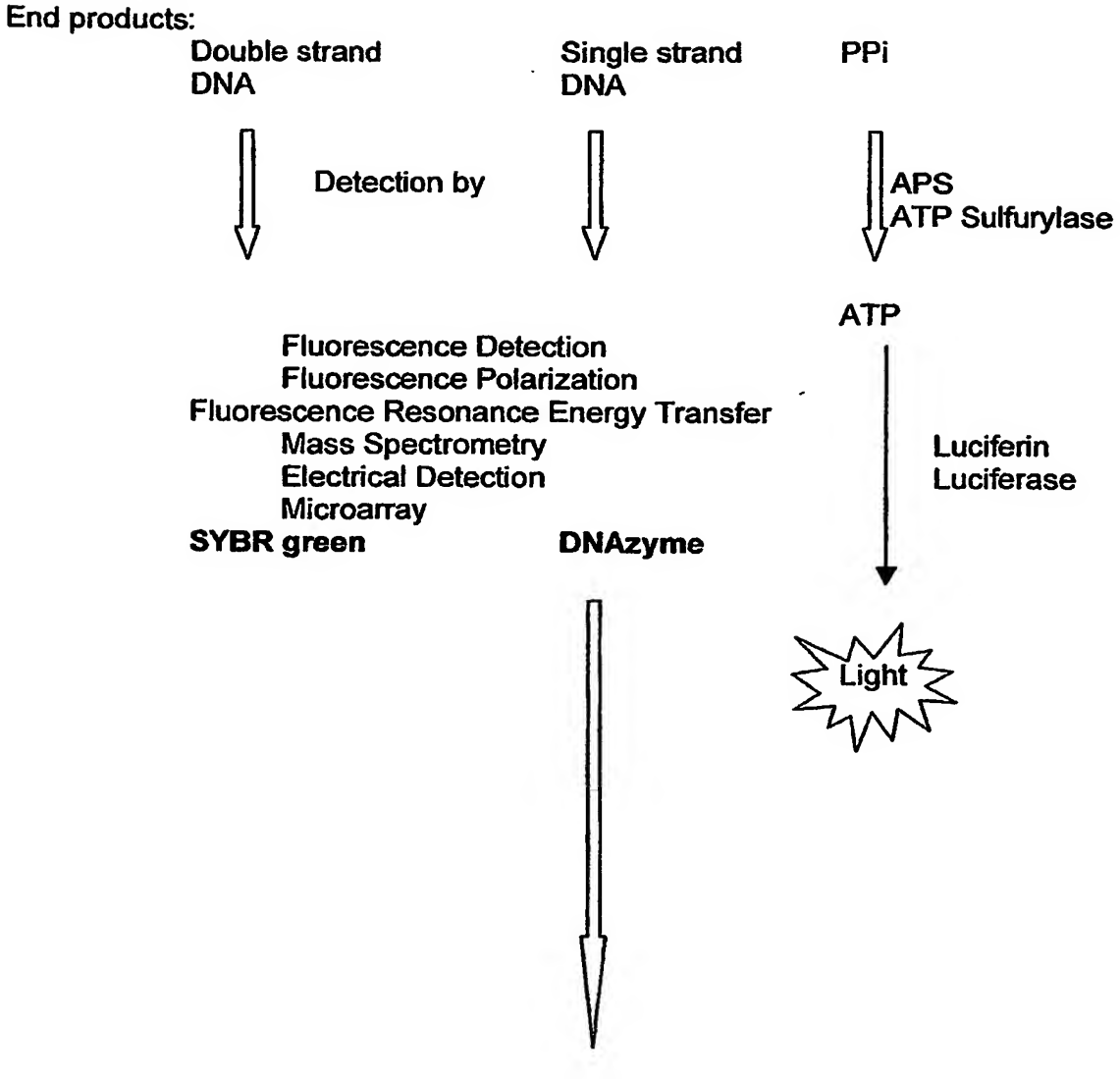

End products:
Double strand DNA
Single strand DNA
PPi
Detection by
APS
ATP Sulfurylase
ATP
Fluorescence Detection
Fluorescence Polarization
Fluorescence Resonance Energy Transfer
Mass Spectrometry
Electrical Detection
Microarray
SYBR green
DNAzyme
Luciferin
Luciferase
Light

Fig. 9

DNAzyme detection of end products - single stranded PPS

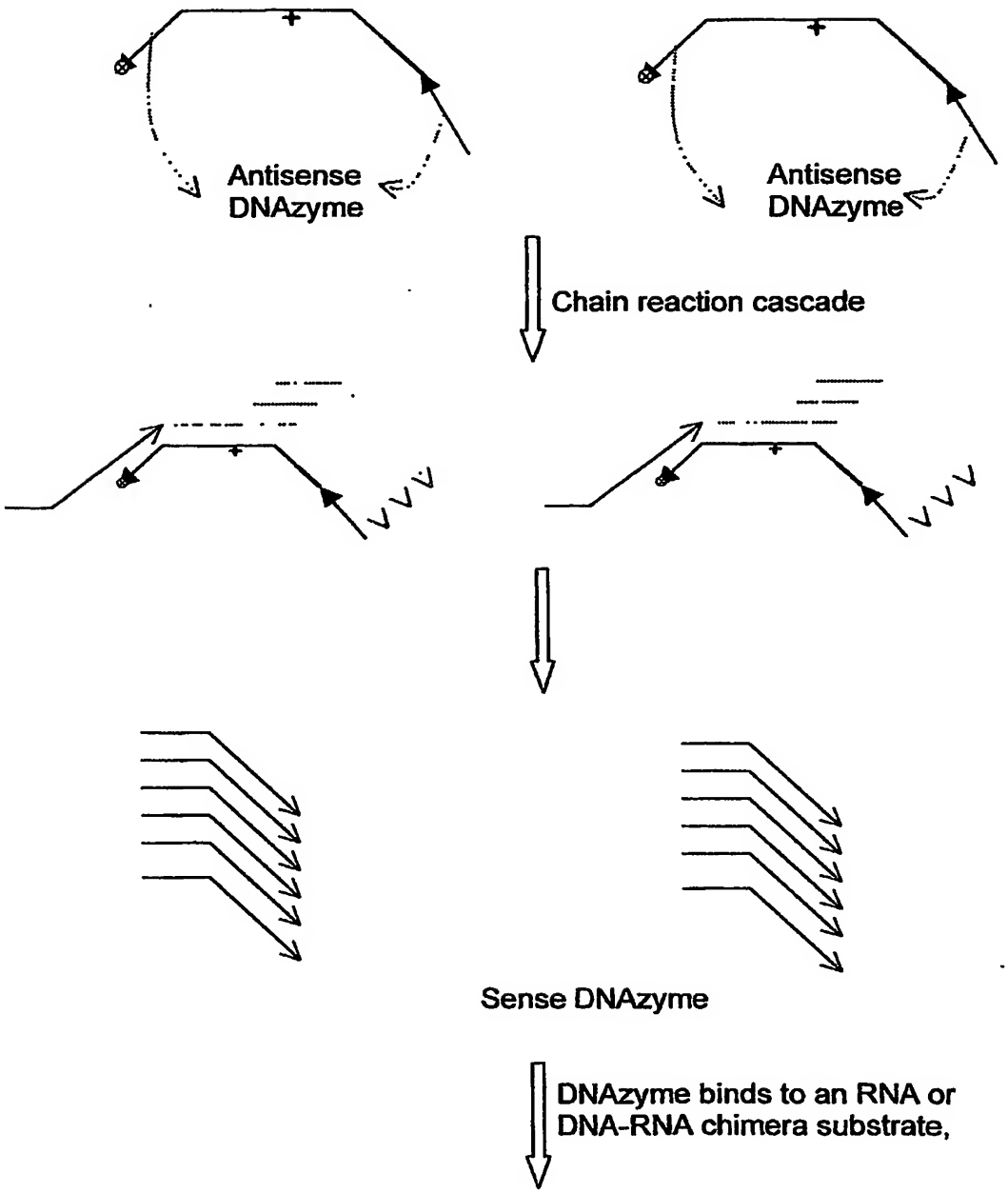


Fig. 10 (continuing)

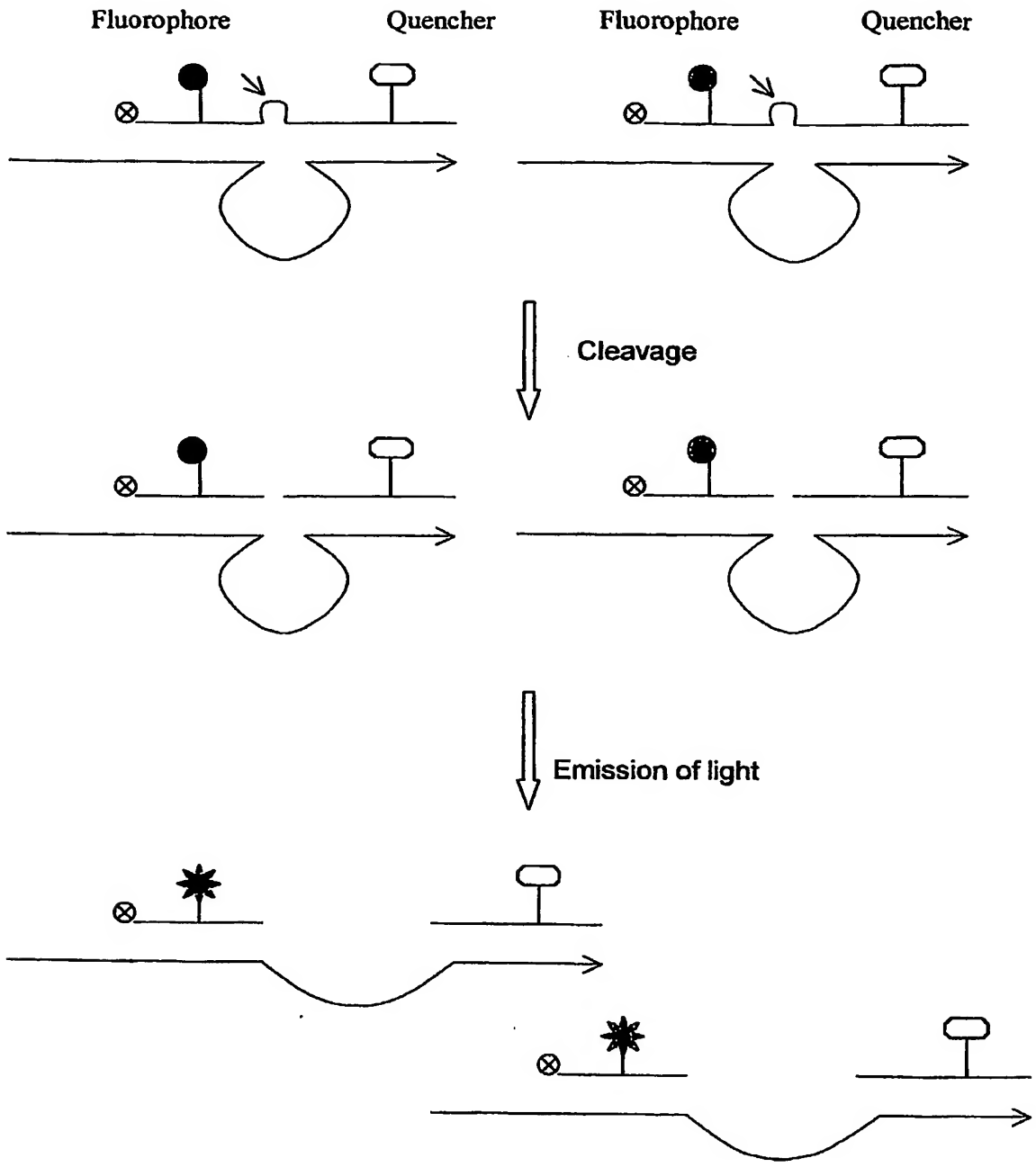

Fluorophore
Quencher
Fluorophore
Quencher
Cleavage
Emission of light

Fig. 10